Pacific Science (1989), vol. 43, no. 1

Mycorrhizal Associations of Selected Plant Species from San Miguel Island, Channel Islands National Park, California[1]

RICHARD E. KOSKE[2] AND WILLIAM L. HALVORSON[3]

ABSTRACT: Vesicular–arbuscular mycorrhizae (VAM) were detected in six native plant species: *Camissonia cheiranthifolia* ssp. *cheiranthifolia*, *Coreopsis gigantea*, *Distichlis spicata*, *Dudleya greenei*, *Eriogonum grande* ssp. *rubescens*, and *Lavatera assurgentiflora*. Levels of root colonization were greater in November than in July. No mycorrhizae were apparent in plants of *Cakile maritima*, *Mesembryanthemum crystallinum*, or *M. nodiflorum*. A total of ten species of VAM fungi, three of them undescribed, were recovered from root zones of *Dudleya*, *Coreopsis*, and *Lavatera*. Genera of fungi included *Entrophospora*, *Glomus*, and *Scutellospora*. The reinvasion of barren areas of the island from which some native plant species were extirpated by overgrazing and erosion may be dependent upon the reestablishment of a population of VAM fungi.

SAN MIGUEL ISLAND (Figure 1) has had a long history of disturbance from ranching and military activities (Roberts 1978). Loss of vegetation from more than a century of grazing by sheep, whose damage was accentuated by periodic droughts and the nearly constant wind-scouring of the island, has led to the formation of large denuded areas [c. 37 km^2, 15% of the habitable island surface; Johnson (1980)]. This has resulted in the reduction of the distribution of many native plant species, which are now scattered on steep slopes and in inaccessible canyons. Also, alien species are now dominant over much of the land that was not denuded. A long-term research project is underway to determine the characteristics and dynamics of the plant assemblages on the island as they recover from this disturbance. Recovery has been in progress since the National Park Service (NPS) began carrying out a program of conservation management in 1974. At present, the floral list includes 233 species, of which 75.1% are native (5.6% Channel Islands endemics) and 24.9% are alien (Halvorson, 1989).

Other studies have suggested that vesicular–arbuscular mycorrhizae (VAM) may be of critical importance in the reinvasion of disturbed sites by extirpated plant species (Janos 1980; Miller 1979, 1987; Reeves et al. 1979). Approximately 50% of angiosperm species appear to have an absolute requirement for forming the VAM type of association (Trappe 1987). Since all the VAM fungi apparently are obligate symbionts they are absent from soils that have been unvegetated for long periods of time (Harley and Smith 1983); such is the case in the denuded areas of San Miguel Island. The recovery of a disturbed community to its earlier, undisturbed state can be slowed or prevented by the absence of VAM fungi from that site, resulting in a taxonomically different community composed of species that do not require the association (Janos 1980; Miller 1979, 1987; Reeves et al. 1979). In addition, the presence of VAM fungi in a soil may affect the outcome of competition between plant species that require VAM and those that do not (Janos 1980). Therefore, the ability of extirpated species to reinvade areas vegetated by other species may depend on the mycorrhizal requirements of each of the plant species and

[1] This project was partially funded by the National Park Service Science Program. Manuscript accepted 30 January 1988.

[2] University of Rhode Island, Department of Botany, Kingston, Rhode Island 02881.

[3] Channel Islands National Park, 1901 Spinnaker Drive, Ventura, California 93001.

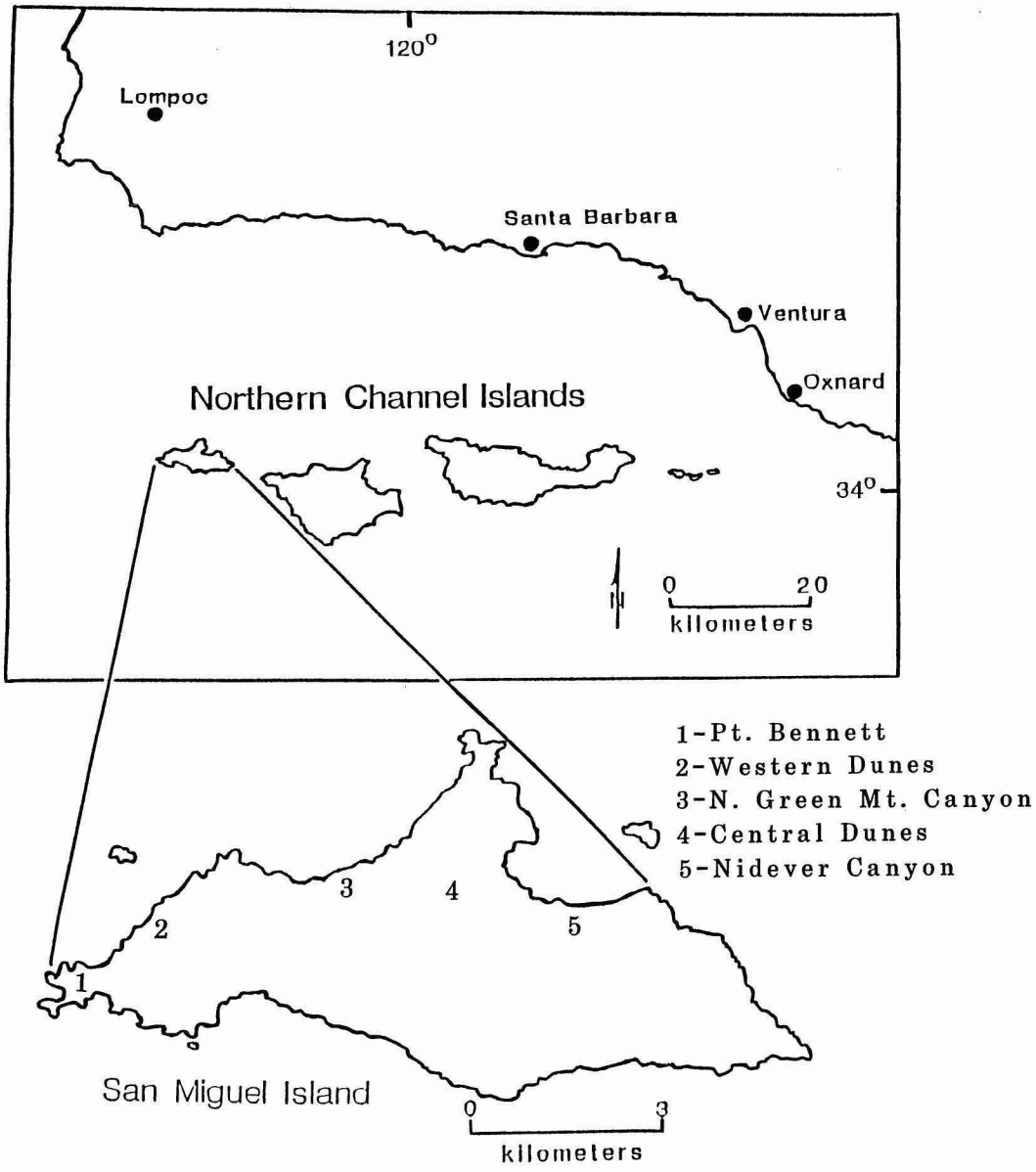


FIGURE 1. Location of San Miguel Island and collection sites.

on the presence of VAM fungi (Miller 1979, 1987; Reeves et al. 1979).

On San Miguel Island, removal of sheep in the 1950s has permitted the reinvasion of some disturbed sites by species formerly restricted to a small refugia. An understanding of the mycorrhizal requirements of these species is needed, however, in order to understand the potential rate at which these small populations may grow and expand into barren sites and areas presently colonized by alien species.

The purpose of this study was to assess the mycorrhizal status of these reinvading species

and species that are widespread on the island. Such information is important to the understanding of population dynamics and to the success of attempts at habitat restoration. We selected four native species whose distributions are expanding, *Coreopsis gigantea* (Kell.) Hall., *Dudleya greenei* Rose, *Eriogonum grande* Greene ssp. *rubescens* (Greene) Munz, and *Lavatera assurgentiflora* Kell., to determine the extent of mycorrhizal development. The mycorrhizal status of these plants was compared to two native species widely distributed on the island, *Camissonia cheiranthifolia* (Hornem ex Spreng.) Raimann in Engl. & Prantl. ssp. *cheiranthifolia* Raven and *Distichlis spicata* L., and to three widely distributed alien species that frequently colonize barren areas, *Cakile maritima* Scop., *Mesembryanthemum crystallinum* L., and *M. nodiflorum* L.

STUDY AREA

San Miguel is a 4000-ha island off the coast of southern California. It is the westernmost of the northern Channel Islands, lying about 45 km south of Point Conception and 100 km west–northwest of Ventura. Bedrock on the island is composed primarily of Cretaceous and early-to-mid-Tertiary conglomerates, sandstones, siltstones, shales, and volcanics. Structurally, the island represents the north flank of a folded and faulted anticline whose axis trends northwest–southeast (Johnson 1979, Weaver et al. 1969). Much of the island is covered with sand, both stabilized and unstabilized. Soils of the upland areas are of the vertisol group with high amounts of expandable clays and shrink–swell characteristics (Johnson 1979). The island has a Mediterranean type of climate with distinct wet winter and dry summer periods. The summer, while having little rain, is characterized by heavy and long-lasting fogs, providing a minimum amount of moisture. Rainfall is in the range of 330–355 mm per year, and the mean annual temperature is 13.7°C, with an annual range of 3°C. The island is also very windy, with an annual average of 25 km/hr. The island vegetation is low-growing, with trees (willows) confined to deep drainages. The major communities are grassland, *Haplopappus* scrub, coastal sage scrub, coastal bluff, and coastal dune.

MATERIALS AND METHODS

Root and soil samples were collected in July and November 1985 (Table 1). Care was taken to exclude roots of neighboring species. Roots were fixed in the field in a solution of formalin, acetic acid, ethanol, and water (in a ratio of 2 : 1 : 5 : 7). In the laboratory, roots were cleared and stained using a a modification of the methods of Bevege (1968) and Phillips and Hayman (1970). The fixed roots were cleared by autoclaving for 3 min in 10% KOH. If still dark, they were bleached in 60% Chlorox (c. 3% NaOCl) until straw yellow. Cleared roots were rinsed in a dilute HCl solution, and mycorrhizae were stained by autoclaving the roots for 3 min in 0.05% trypan blue in a solution of lactic acid, glycerol, and water (in a ratio of 1 : 2 : 1). Roots were destained by autoclaving for 3 min in the lactic acid, glycerol and water solution lacking trypan blue.

The extent of colonization of roots by VAM fungi was determined by estimating the percentage (to the nearest 10%) of the length of the absorbing root system that contained arbuscules, vesicles, hyphal coils, or internal hyphae of VAM fungi.

To determine the species of VAM fungi associated with roots of *Lavatera*, *Dudleya*, and *Eriogonum*, soil samples (c. 500 cm^3) mixed with root fragments were collected at a depth of 5–15 cm beneath the soil surface when root samples were collected (Table 1). A 75-cm^3 subsample, composed of 20–30 smaller subsamples withdrawn from each 500-cm^3 sample, was processed to recover spores. Spores were extracted from the soil by a water–sucrose centrifugation technique (Walker et al. 1982). Following centrifugation, spores were collected on a 5.5-cm filter paper (Whatman no. 1) in a Buchner funnel. The filter paper was examined at 30 × with a dissecting microscope, and spores were removed, mounted in a polyvinyl alcohol mountant (Koske and Tessier 1983), and identified with the aid of a compound microscope at 400–1000 × . Identifications were confirmed by comparison with type or authenticated specimens and by con-

TABLE 1

COLLECTION INFORMATION AND EXTENT OF MYCORRHIZAE IN ROOT SYSTEMS OF PLANTS FROM SAN MIGUEL ISLAND

PLANT	COLONIZATION* RANGE	COLONIZATION* MEAN	COLLECTION DATE	NO.†	LOCATION
Native species with expanding ranges					
Coreopsis	30–50	40	VII 85	3/ 3	N. Green Mt. Canyon
Dudleya	0–10	5	XI 85	4/2	Nidever Canyon
Eriogonum	0–90	70	VII 85 XI 85	7/4	Western Dunes
Lavatera	20–90	70	VII 85	10/10	Pt. Bennett
Native species with widespread ranges					
Camissonia	0–90	45	VII 85	4/3	Central Dunes
Distichlis	50–90	73	VII 85	3/3	Central Dunes
Alien species with widespread ranges					
Cakile	0	0	VII 85	5/0	Western Dunes
Mesembryanthemum crystallinum	0	0	VII 85	3/0	Pt. Bennett
M. nodiflorum	0	0	VII 85	3/0	Pt. Bennett

* Percentage of root length colonized by VAM fungi.
† Number of samples per site/Number of samples with mycorrhizae.

TABLE 2

VAM FUNGI ASSOCIATED WITH SELECTED PLANTS ON SAN MIGUEL ISLAND

VAM FUNGUS	HOST PLANT *Lavatera*	HOST PLANT *Dudleya*	HOST PLANT *Eriogonum*
Entrophospora infrequens (Hall) Ames & Schneider	+	+	
Glomus aggregatum Schenck & Smith	+	+	
G. etunicatum Beckec & Gerd.	+	+	+
G. intraradices Schenck & Smith	+	+	+
G. microaggregatum Koske, Gemma & Olexia		+	
G. monosporum Gerd. & Trappe	+	+	+
G. pansihalos Berch & Koske			+
G. 601*	+	+	
Scutellospora calospora (Nicol. & Gerd.) Waker & Sanders		+	
S. 581†		+	

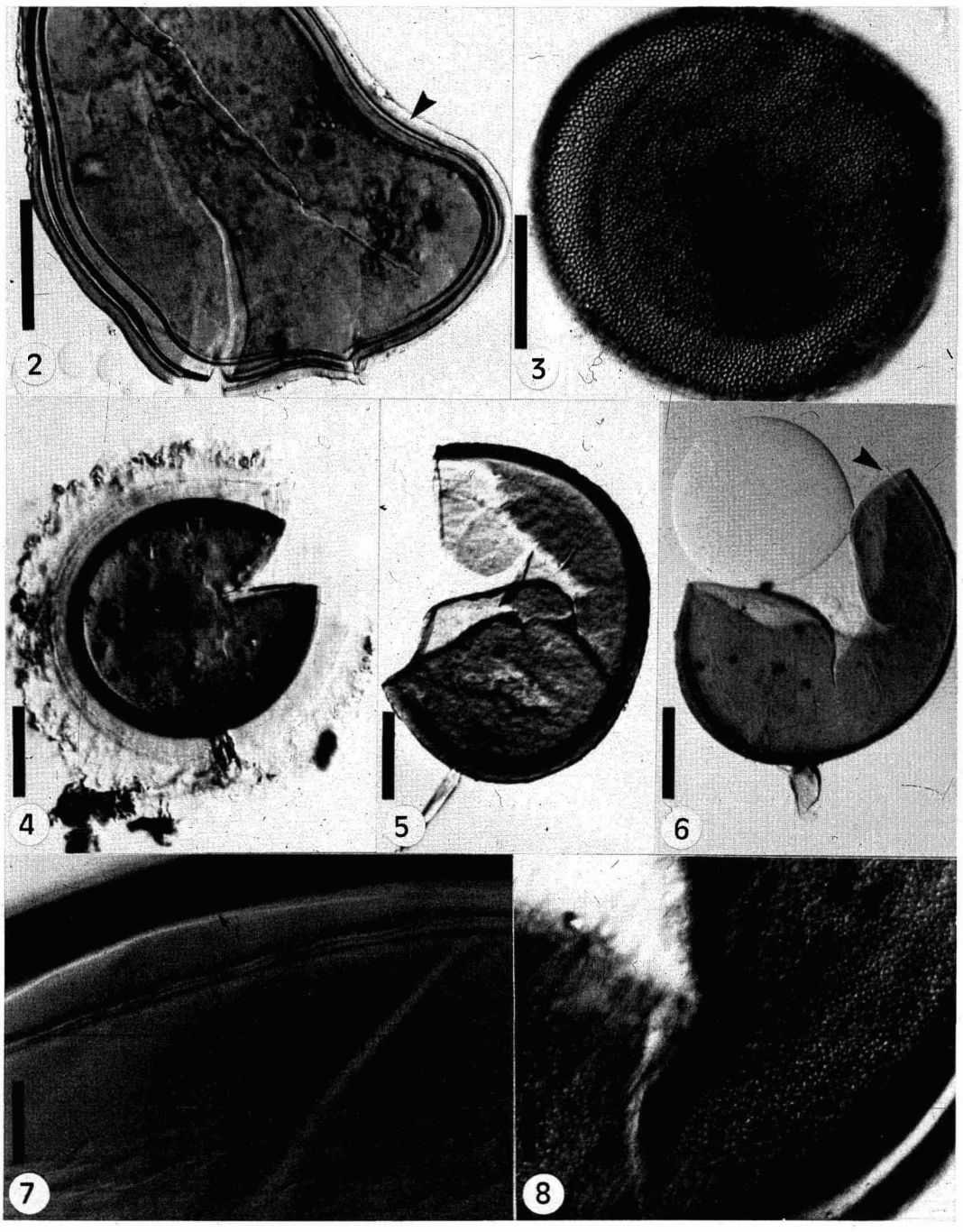
* *Glomus* 601: spore pale yellow to pale brownish-yellow, globose to subglobose, 80–100 × 80–130 μm; spore wall structure of three walls, outermost wall pale, 1–2 μm thick, ephemeral; middle wall pigmented, 2–8 μm thick, laminated; innermost wall pale, <1 μm thick, membranous, continuing down attachment hypha; attachment hypha pale, 6–10 μm broad.
† *Scutellospora* 581: spores orange-brown, globose to irregular, 160–310 × 120–310 μm; spore wall structure of three walls, outermost wall brittle, orange-brown, 4–13 μm thick; middle wall hyaline, flexible, 1–3 μm thick; inner wall hyaline, amorphorus, of variable thickness.

sultation with other VAM fungal taxonomists. Voucher specimens have been deposited in the mycological herbarium at the University of Rhode Island.

Soil pH was determined with a Fisher Accumet pH meter by placing the electrode in the liquid phase of a slurry of soil mixed with 0.01 *M* $CaCl_2$ after a 1-hr equilibration (Schofield and Taylor 1955).

RESULTS AND DISCUSSION

Ten species of VAM fungi were recovered from the soil samples (Table 2, Figures 2–8).



FIGURES 2–8. Crushed spores of VAM fungi from San Miguel Island. *2*, *Glomus intraradices*, note pale outermost wall (arrow) and two darker laminations of innermost wall (bar = 50 μm); *3*, *Entrophospora infrequens*, ornamentations of outermost spore wall (bar = 50 μm); *4*, *Glomus pansihalos*, note thick, pale outer wall and darker, laminated wall (bar = 50 μm); *5*, *Glomus etunicatum* (bar = 50 μm); *6*, *Scutellospora* 581, note wrinkling innermost wall grouping (arrow) (bar = 200 μm); *7*, *Glomus pansihalos*, warty surface of laminated wall (bar = 20 μm); *8*, *Scutellospora* 581, walls 1–4 are indicated, note heavy wrinkling of wall 4 (arrow) (bar = 10 μm).

The root zones of *Dudleya*, *Lavatera*, and *Eriogonum* contained 9, 6, and 4 species of VAM fungi, respectively. Five of the species, *Scutellospora calospora*, *Glomus etunicatum* (Figure 5), *G. intraradices* (Figure 2), *G. monosporum*, and *Entrophospora infrequens* (Figure 3), have been previously isolated from the southwestern United States (Ames and Schneider 1979, Bethlenfalvay et al. 1984, Bloss and Walker 1987, Nemec et al. 1981). Both *E. infrequens* and *G. monosporum* occurred in agricultural areas in Ventura County, adjacent to Santa Barbara County in which San Miguel Island is located (Ames and Schneider 1979, Nemec et al. 1981). Of the other VAM fungal species recovered, *G. pansihalos* (Figures 4, 8), *G. aggregatum*, and *G. microaggregatum* are known from sand dunes in Michigan and along the Atlantic Coast of the United States (Berch and Koske 1986, Koske 1985, Koske et al. 1986). The latter two species also occur in Hawaiian sand dunes (Koske 1988).

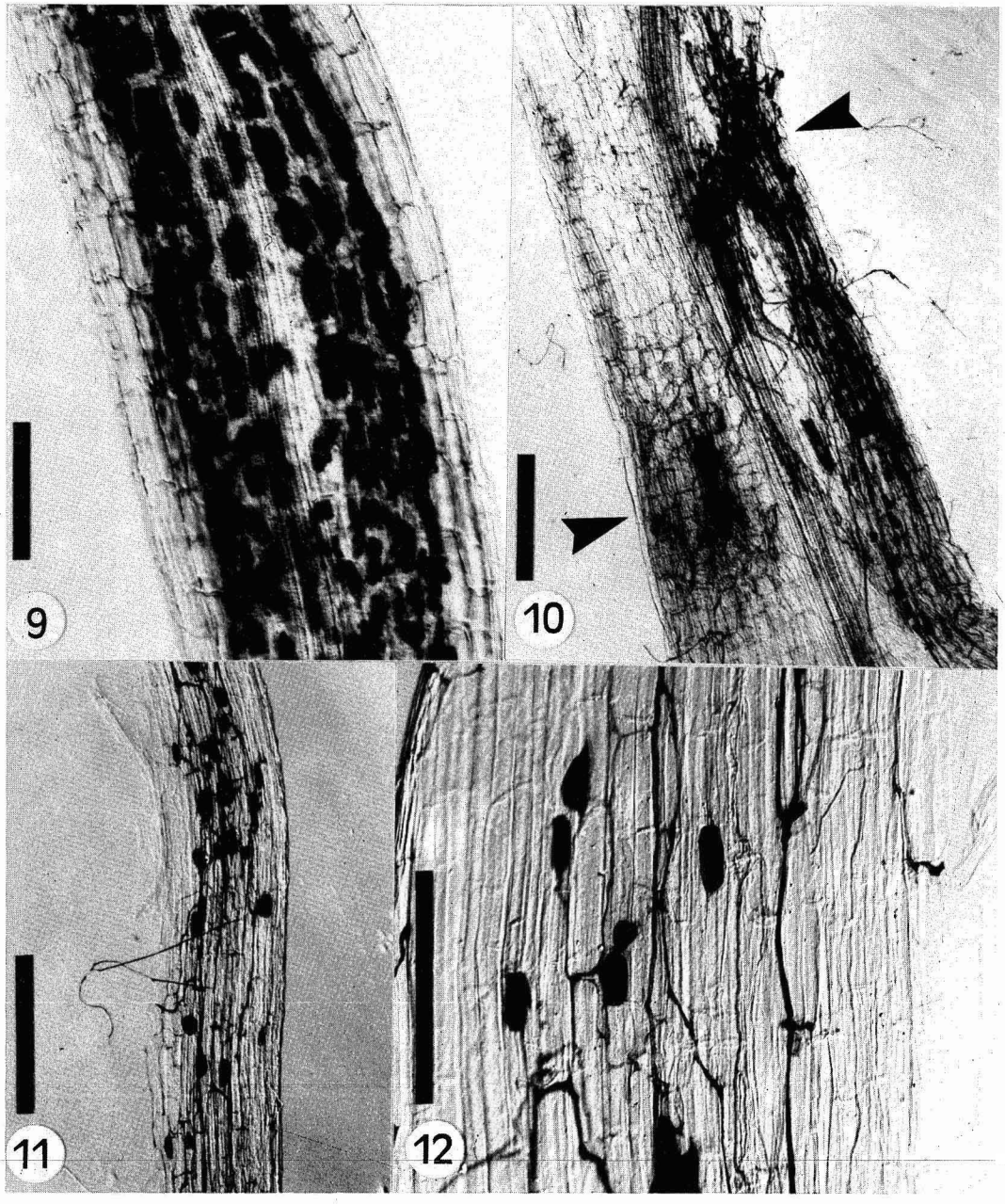
Two of the VAM species, *Scutellospora* 581 (Figures 6, 7) and *Glomus* 601, could not be identified. Descriptions of the first are in preparation. Spores of *Glomus* 601 resemble those of *G. diaphanum* Morton & Walker (1984) in size, features of the attachment hypha, and wall structure, but differ in having a pale-yellow to brownish-yellow color and in possessing an ephemeral outermost wall 1–2 μm thick.

The six genera of native plants were mycorrhizal, although not every specimen possessed VAM at the time of sampling (Table 1). None of the alien species was mycorrhizal, supporting the observation that members of the Aizoaceae and Brassicaceae routinely lack VAM (Gerdemann 1968). Vesicular–arbuscular mycorrhizae colonization levels were greatest in *Lavatera* and *Distichlis*, and many root segments of the former were densely colonized, with most cortical cells containing arbuscules (Figure 9). The *Coreopsis* root systems were also mycorrhizal (Figure 10). Collections of *Dudleya* and *Eriogonum* included some root systems that lacked VAM. The level of colonization was very low in *Dudleya* (max. colonization = 10%), and two of the plants were nonmycorrhizal. The three *Eriogonum* samples collected in July had 80–90% colonization (Figures 11, 12), while the November collections of this species showed only 0–10% colonization. The July samples contained abundant arbuscules, and nearly every cortical cell was occupied in some root segments.

The low levels of colonization present in both November collections (all the *Dudleya* plants and four of the *Eriogonum* plants) may be indicative of seasonal fluctuations in VAM intensity. These collections were made at the beginning of the growing season on San Miguel Island when root extension rates were high. Colonization of roots by VAM fungi typically is very low or absent during the first few weeks of rapid root growth following emergence, and levels of colonization reach a maximum when growth of root systems slows at the end of the growing season (Gemma 1987, Sutton 1973,).

Members of the Polygonaceae frequently are nonmycotrophic (Gerdemann 1968), but exceptions are not uncommon, and both *Eriogonum fasciculatum* Benth. and *E. nodosum* Small formed VAM in Anza Borrego State Park, a desert site in southern California (Bethlenfalvay et al. 1984).

Results from the assessment of the mycorrhizal status of the extirpated and widespread native species and of the alien species suggest that the reinvasion of barren areas and restoration of plant communities on San Miguel Island may be dependent upon the reestablishment of a population of VAM fungi. Plant species in which all individuals sampled possessed mycorrhizae (*Coreopsis*, *Distichlis*, and *Lavatera*) are putative obligate mycotrophs, having an absolute requirement for VAM to complete their life cycles (Janos 1980, Trappe 1987), although additional sampling and experimentation are needed to confirm this assumption. Such species would compete poorly against facultative mycotrophs (*Dudleya*, *Camissonia*, and *Eriogonum*) and nonmycotrophs (*Cakile* and *Mesembryanthemum* spp.) in barren sites lacking VAM fungi (Janos 1980). The addition of VAM fungi to soil in certain sites may be necessary to restore the plant communities to their natural condition.



FIGURES 9–12. Vesicular–arbuscular mycorrhizae of three native plant species. *9*, *Lavatera assurgentiflora*, darkly stained arbuscules in nearly every cortical cell (bar = 100 μm); *10*, *Coreopsis gigantea*, two areas of root densely colonized by VAM fungi (arrows) (bar = 500 μm); *11,12*, *Eriogonum grande*, darkly staining vesicles and runner hyphae (bars = 100 μm in Figure 11 and 50 μm in Figure 12).

ACKNOWLEDGMENTS

We thank Joe Morton and Chris Walker for taxonomic assistance.

LITERATURE CITED

AMES, R. N., and R. W. SCHNEIDER. 1979. *Entrophospora*, a new genus in the Endogonaceae. Mycotaxon 8:347–352.

BERCH, S. M., and R. E. KOSKE. 1986. *Glomus pansihalos*, a new species in the Endogonaceae. Mycol. 78:832–836.

BETHLENFALVAY, G. J., S. DAKESSIAN, and R. S. PACOVSKY. 1984. Mycorrhizae in a southern California desert: Ecological implications. Can. J. Bot. 62:519–524.

BEVEGE, D. I. 1968. A rapid technique for clearing tannins and staining roots for detection of mycorrhizas caused by *Endogone* spp., and some records of infection in Australasian plants. Trans. Brit. Mycol. Soc. 51:808–810.

BLOSS, H. E., and C. WALKER. 1987. Some endogonaceous mycorrhizal fungi of the Santa Catalina Mountains in Arizona. Mycol. 79:649–654.

GEMMA, J. N. 1987. Physiological ecology of VA mycorrhizal fungi in sand dunes. Ph.D. Thesis. University of Rhode Island, Kingston.

GERDEMANN, J. W. 1968. Vesicular–arbuscular mycorrhizae and plant growth. Ann. Rev. Phytopathol. 6:397–418.

HALVORSON, W. L. 1989. Alien plant species at Channel Islands National Park. *In* C. P. Stone, ed. Control of introduced plants in Hawaii's native ecosystems. National Park Service, Coop. Park Studies Unit, University of Hawaii. (in press)

HARLEY, J. L., and S. E. SMITH. 1983. Mycorrhizal symbiosis. Academic Press, London.

JANOS, D. P. 1980. Mycorrhizae influence tropical succession. Biotropica 12:56–64.

JOHNSON, D. L. 1979. Geology, soils, and erosion. Pages 3.1–3.12 *in* D. M. Power, ed. Natural resources study of the Channel Islands National Monument, California. Santa Barbara Museum of Natural History, Santa Barbara, Ca.

———. 1980. Episodic vegetation stripping, soil erosion, and landscape modification in prehistoric and recent historic times, San Miguel Island, California. Pages 103–121 *in* D. M. Power, ed. The California Islands: Proceedings of a multidisciplinary symposium. Santa Barbara Museum of Natural History, Santa, Santa Barbara, Ca.

KOSKE, R. E. 1985. *Glomus aggregatum* amended, a distinct taxon in the *Glomus fasciculatum* complex. Mycol. 77:619–630.

———. 1988. VA mycorrhizae of some Hawaiian dune plants. Pac. Sci. 42:217–229.

KOSKE, R. E., and B. TESSIER. 1983. A convenient, permanent slide mounting medium. Mycol. Soc. Amer. Newsl. 34(2):59.

KOSKE, R. E., J. N. GEMMA, and P. D. OLEXIA. 1986. *Glomus microaggregatum*, a new species in the Endogonaceae. Mycotaxon 26:125–132.

MILLER, R. M. 1979. Some occurrences of vesicular–arbuscular mycorrhizae in natural and disturbed ecosystems of the Red Desert. Can. J. Bot. 57:619–623.

———. 1987. The ecology of vesicular–arbuscular mycorrhizae in grass- and shrublands. Pages 135–170 *in* G. R. Safir, ed. Ecophysiology of VA mycorrhizal plants. CRC Press, Boca Raton, Fla.

MORTON, J. B., and C. WALKER. 1984. *Glomus diaphanum*: A new species in the Endogonaceae common in West Virginia. Mycotaxon 21:431–440.

NEMEC, S., J. A. MENGE, R. G. PLATT, and E. L. V. JOHNSON. 1981. Vesicular–arbuscular mycorrhizal fungi associated with citrus in Florida and California and notes on their distribution and ecology. Mycol. 73:112–127.

PHILLIPS, J. M., and D. S. HAYMAN. 1970. Improved procedures for clearing roots and staining parasitic and vesicular–arbuscular mycorrhizal fungi for rapid assessment of infection. Trans. Brit. Mycol. Soc. 55:158–161.

REEVES, F. B., D. WAGNER, T. MOORMAN, and J. KIEL. 1979. The role of endomycorrhizae in revegetation practices in the semi-arid west. I. A comparison of incidence of mycorrhizae in severely disturbed vs. natural environments. Amer. J. Bot. 66:6–13.

ROBERTS, L. 1978. Historic resource study—Channel Islands National Monument and San Miguel Island, California. Chambers Consultants and Planners, Santa Barbara, Ca.

SCHOFIELD, R. K., and A. W. TAYLOR. 1955. The measurement of soil pH. Soil Sci. Soc. Amer. Proc. 19:164–167.

SUTTON, J. C. 1973. Development of vesicular–arbuscular mycorrhizae in crop plants. Can. J. Bot. 51:2487–2493.

TRAPPE, J. M. 1987. Phylogenetic and ecologic aspects of mycotrophy in the angiosperms from an evolutionary standpoint. Pages 5–26 *in* G. R. Safir, ed. Ecophysiology of VA mycorrhizal plants. CRC Press, Boca Raton, Fla.

WALKER, C., C. M. MIZE, and H. S. MCNABB. 1982. Populations of endogonaceous fungi in two locations in central Iowa. Can. J. Bot. 60:2518–2529.

WEAVER, D. W., D. P. DOERNER, and B. NOLF, eds. 1969. Geology of the northern Channel Islands and southern California borderland. Pacific Sections, Amer. Petrol. Geol. and Soc. Econ. Paleontol. and Mineral., Spec. Publ.